The Problem of Falsely Doubtful and Positive Reactions in the Serology of Syphilis*

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AT no time more than at present has the problem of falsely doubtful and positive reactions in the serology of syphilis commanded as much attention and importance. This is not only due to legally required serologic tests before marriage and during pregnancy, but to the testing of thousands upon thousands of selectees for the Army, Navy, and other services, as well as of thousands upon thousands of civilian donors of blood for the preparation of plasma.

However, while doubtful or positive reactions occurring in presumably non-syphilitic individuals are always very disturbing and place a heavy responsibility upon physicians in relation to their clinical interpretation, this present widespread employment of serologic tests for syphilis is not without the great advantage of discovering unsuspected cases of the disease. This in turn usually results in appropriate treatment which is not only of benefit to the individuals concerned, but to the public at large by way of reducing the chances of its transmission to others.

Following the discovery of the Wassermann or complement-fixation and various flocculation tests for syphilis, falsely positive reactions were reported from time to time with practically all methods, not only in a large number of various non-syphilitic diseases of human beings but in presumably normal non-syphilitic individuals as well. But up to about fifteen years ago they were thought to be due largely to technical errors, except in the case of leprosy and malaria. Since then, however, and especially during the past eight years, the results of annual interstate serologic surveys conducted by the U. S. Public Health Service in coöperation with the American Society of Clinical Pathologists, have clearly shown that biologic falsely positive reactions may occur not only in a number of non-syphilitic diseases and conditions but in presumably normal non-syphilitic individuals as well. I refer especially to these serologic surveys because the Committee on Evaluation of Serodiagnostic Tests for Syphilis have not only selected syphilitic and presumably non-syphilitic donors with commendable care and skill, but because all specimens were submitted to various author-serologists themselves in whose laboratories it may be reasonably assumed that technical errors were reduced to a minimum. For these reasons, therefore, I have largely confined myself in this study to an analysis of the results reported by author-serologists and state laboratories in the

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TABLE 1

Sources of Technical Falsely Doubtful and Positive Serologic Reactions

(A) Due to Errors in Collection Resulting in Contamination or Hemolysis	*(B) Due to Errors in the Laboratory*
Bacterial contamination	Insufficient skill and experience
Chemical contamination from unclean containers	Failure to follow technic exactly as described by author-serologists
Using oxalate or citrate	Mix-up of specimens
Improper storage of specimens	Improperly prepared glassware
Delay in sending specimens to laboratory	Faulty reagents
Mislabelling of specimens	Inexact measurements
Post-mortem specimens	Inadequate controls
	Improper reading of reactions
	Errors in recording and reporting reactions

nine serologic surveys begun in 1935, including the Washington serology conference held in 1941.

DOUBTFUL REACTIONS

While the committee has adopted the plan of penalizing laboratories one-half point for a doubtful reaction occurring in a presumably non-syphilitic individual and crediting one-half point for a doubtful reaction in the case of a syphilitic individual, in this study I have preferred to list doubtful reactions on the same basis as positive reactions, as was the policy of the committee in the first survey held in 1935. In practice at least it has been my experience that doubtful reactions occurring in presumably non-syphilitic individuals require almost as much consideration from the standpoint of clinical interpretation and management as weakly positive ones, and especially if repeatedly doubtful. Furthermore, while single doubtful reactions should never alone be the basis for the diagnosis of syphilis, neither should they be completely ignored because the disease is unexpected, and especially since they are sometimes the only evidence indicative of possible chronic latent syphilis, either congenital or acquired.

TECHNICAL FALSELY DOUBTFUL AND POSITIVE REACTIONS

Unfortunately there are many sources for falsely doubtful and positive reactions due to technic. As summarized in Table 1, these may be divisible into those incident to the collection of blood resulting in excessive contamination or hemolysis, and thereby usually attributable to the physician himself, and those attributable to faulty technic in the laboratory. Certainly all serologic tests, regardless of their technical simplicity, require skill, experience, and scrupulous attention to all details regardless of how inconsequential they may appear. For this reason the Committee on the Evaluation of Serodiagnostic Tests for Syphilis has always placed proper emphasis on the advisability of technicians conducting the tests exactly as described by their author-serologists. And, needless to state, the incidence of technical falsely doubtful and positive reactions varies according to the skill with which the tests are conducted; consequently, no test can be better than the laboratory conducting it. Under the circumstances it is to be expected that the incidence of falsely doubtful and positive reactions due to errors in technic will increase under present conditions because of the large number of technicians with insufficient training and experience being rushed into serologic testing for syphilis.

But there is another angle to this subject which has not commanded as much attention as it deserves. I refer

to the possibility of technical errors being inherent in the test itself so that falsely doubtful and positive reactions may occur in the hands of skillful and experienced technicians and, indeed, in those of the author-serologist himself. As is well known, every serologic test now employed can be made so sensitive as to yield doubtful or positive reactions with the sera of normal non-syphilitic individuals. In the race for sensitivity in the detection of syphilis author-serologists may commit the error of making their tests too sensitive. For my own part, I always have been and continue to be dedicated to the principle that it is better to reduce technical sensitivity to the level of 100 per cent negative reactions with the sera of normal non-syphilitic individuals even though this inevitably means missing the serological detection of an occasional case of chronic latent syphilis. In other words, I believe that the serologic tests for syphilis should be made technically only as sensitive as is consistent with what may be termed "practical specificity" for syphilis.

Under the circumstances it is not at all easy to determine what percentage of falsely doubtful and positive reactions is due to errors in the collection of blood and the setting up of tests and that due to the tests themselves. But if it may be assumed that in the serologic surveys the tests conducted in the laboratories of author-serologists were set up exactly as they have been described, it is apparent, as shown in Table 2, that under such conditions only one of the ten tests now commonly employed has had, up to the present time, 100 per cent negative reactions in all of the nine serologic surveys with the sera of normal, presumably non-syphilitic, individuals. In this connection, however, I point with pride to the fact that in the 1943 serologic survey all of the author-serologists listed in Table 2 reported 100 per cent negative reactions with the sera of normal, presumably non-syphilitic, individuals.

TABLE 2

Doubtful and Positive Reactions Reported by Author-Serologists with the Sera of Normal Presumably Non-syphilitic Individuals in the Serologic Surveys *

Tests	*Total Sera Tested*	*Doubtful and Positive Reactions*	
		No. Sera	*Per cent*
Eagle Wassermann	850	5	0.6
Eagle macroflocc.	907	13	1.4
Eagle microflocc.	580	11	1.9
Hinton regular	956	5	0.5
Kahn presumptive	967	3	0.3
Kahn standard	1,116	2	0.2
Kline diagnostic	1,116	4	0.4
Kline exclusion	802	8	1.0
Kolmer	1,116	0	0.0
Mazzini slide flocc.	429	1	0.2

* 1935, 1936, 1937, 1938, 1939, 1941, 1942, 1943, and Washington serology conference of 1941

In Tables 3 and 4 it is shown that technical errors alone, apart from those inherent in various complement-fixation and flocculation tests themselves, have apparently occurred in seven of the serologic surveys in a disturbingly large number of state laboratories. Thanks to the excellent efforts of the Committee on the Evaluation of the Serodiagnostic Tests for Syphilis, however, the situation in the last survey (1943) was greatly improved over that of the first, conducted in 1936. For example, in 1935, 50 per cent of state laboratories reported falsely doubtful and positive reactions with various complement-fixation tests, but only 13.4 per cent in 1943 (Table 3). Furthermore, the percentage of these reactions has dropped from between 1.0 and 10.0 per cent in 1936, to between 0.7 and 0.8 per cent in 1943. Somewhat less but nevertheless definite improvement has been observed also in the case of the various flocculation tests (Table 4). Thus, while 67.9 per cent of laboratories reported falsely doubtful and positive reactions in 1936, only 29.7 per cent reported them in 1943. And while the incidence of these reactions varied from 1.0 to 40.0 per cent in 1936, it

TABLE 3

Negative, Doubtful and Positive Complement-fixation Reactions Conducted by Various Methods Reported by State Laboratories with the Sera of Normal Presumably Non-syphilitic Individuals in the Serologic Surveys

Year	Total Labs.	100 Per cent Negative Reactions		Doubtful and Positive Reactions		
		No. Labs.	Per cent Labs.	No. Labs.	Per cent Labs.	Per cent Reactions
1936	22	11	50	11	50	1.0 to 10.0
1937	35	22	63	13	37	0.5 to 8.9
1938	33	23	70	10	30	1.0 to 10.0
1939	26	15	60	11	40	0.9 to 6.0
1941	31	23	74.2	8	25.8	0.8 to 1.5
1942	28	20	71.4	8	28.6	0.8 to 0.9
1943	30	26	86.6	4	13.4	0.7 to 0.8

TABLE 4

Negative, Doubtful and Positive Flocculation Reactions Conducted by Various Methods Reported by State Laboratories with the Sera of Normal Presumably Non-syphilitic Individuals in the Serologic Surveys

Year	Total Labs.	100 Per cent Negative Reactions		Doubtful and Positive Reactions		
		No. Labs.	Per cent Labs.	No. Labs.	Per cent Labs.	Per cent Reactions
1936	28	9	32.1	19	67.9	1.0 to 40.0
1937	39	17	43.3	22	56.7	1.0 to 25.0
1938	62	39	62.9	23	37.1	1.0 to 15.0
1939	51	19	37.3	32	62.7	0.9 to 14.8
1941	56	32	57.1	24	42.9	0.7 to 4.6
1942	72	41	57.0	31	43.0	0.8 to 16.3
1943	74	52	70.3	22	29.7	0.8 to 16.8

dropped to between 0.8 and 16.8 per cent in 1943.

Under the circumstances it is apparent that there is still much room for improvement on the part of some state laboratories if it is assumed that the performance of tests in the laboratories of the author-serologists (Table 2) is acceptable as a measure of the incidence of falsely doubtful and positive reactions with the sera of normal, presumably non-syphilitic, individuals. Fortunately, many state laboratories are conducting serologic surveys themselves as a check on the performance of municipal, hospital, and private laboratories within their confines. I have not sufficient data bearing upon the results observed, but I hazard the opinion that not a few are below the performance of the state laboratories themselves although these intrastate surveys are gradually and progressively improving the situation in a manner analogous to the annual interstate surveys conducted by the Committee on the Evaluation of the Serodiagnostic Tests for Syphilis.

BIOLOGICAL FALSELY DOUBTFUL AND POSITIVE REACTIONS IN NORMAL NON-SYPHILITIC INDIVIDUALS

Biological falsely doubtful and positive reactions are so designated because they are due to the presence of a reagin-like or other substance in the serum (shortly to be discussed), and therefore likely to occur in tests correctly conducted with skill and experience. That this substance may occur in the sera of normal non-syphilitic individuals cannot be denied but the true incidence is still unknown. In 1941 Eagle [1] estimated that with present-day serologic tests the incidence among university students was about 1 for every 4,000 persons tested, and perhaps even less. Mohr and his

colleagues [2] have recently reported 9 normal persons yielding these biologic nonspecific positive reactions.

As previously stated, falsely doubtful and positive reactions with the sera of normal persons may be and doubtless are due in the great majority of instances to technical factors incident to the conduct of the tests or inherent in them. For this reason it is not at all easy to differentiate between those falsely doubtful and positive reactions due to technical factors and those due to the biological factor. In so far as practising physicians are concerned, however, the important matter is the incidence of falsely doubtful and positive reactions due to a summation of these causes. In the 1943 serologic survey about 131 sera were supplied by the committee from normal, presumably non-syphilitic, donors. As previously stated, all of the author-serologists listed in Table 5 reported negative reactions. The same was true of many of the state laboratories participating in the survey but, on the other hand, a few of the latter reported all the way from one doubtful or positive reaction per 100 individuals to as high as one per 6 in the case of a supersensitive flocculation test. I mention these facts because the performance of state laboratories is probably acceptable as a broad and general index of that of laboratories in general. Undoubtedly the great majority and probably all of these falsely doubtful and positive reactions in such a small series of sera were due to technical factors, but the results are sufficient for indicating what physicians may be warned to expect with the serologic tests now commonly employed under what may be regarded as average conditions.

Much less can be stated in regard to falsely doubtful and positive reactions with the cerebrospinal fluids of non-syphilitic individuals. For various reasons it has not been possible for the Committee on the Evaluation of Serodiagnostic Tests for Syphilis to supply them for the annual interstate serologic surveys. In so far as tests conducted by author-serologists themselves are concerned, however, data are available from the 1935 survey and the Washington serology conference held in 1941. These, as summarized in Table 6, indicate that cerebrospinal fluids are much less likely than sera to yield these reactions. Undoubtedly this is due in large part to technical conditions but, on the other hand, it is quite likely that the reagin-like substance that may occur in the sera of normal non-syphilitic individuals yielding falsely doubtful and positive reactions does not occur in the cerebrospinal fluids of normal individuals. At least it is well known that natural antibodies occurring in the sera of normal individuals do not occur in

TABLE 5

Falsely Doubtful and Positive Reactions with Sera Reported by State Laboratories in the 1943 Serologic Survey

Tests	*No. Labs*	*No. Reporting Doubtful Reactions*	*No. Reporting Positive Reactions*	*Per cent Doubtful and Positive Reactions*
Eagle Wassermann	3	0	0	0.0
Eagle macroflocc.	2	1	0	0.8
Eagle microflocc.	2	0	1	0.8
Hinton regular	7	2	3	0.8– 3.2
Kahn presumptive	3	0	1	1.5
Kahn standard	32	3	1	0.8
Kline diagnostic	15	4	4	0.8– 3.1
Kline exclusion	4	2	3	3.1–16.8
Kolmer	18	2	0	0.8– 1.6
Mazzini flocc.	3	1	1	0.8

their cerebrospinal fluids, to which further reference will be made in relation to antibody for spirochetes in normal and syphilitic individuals.

TABLE 6

Falsely Doubtful and Positive Reactions with Cerebrospinal Fluids *

Tests	Total Tested	Doubtful and Positive Reactions	
		No.	Per cent
Eagle Wassermann	106	0	0.0
Eagle macroflocc.	110	0	0.0
Davies-Hinton flocc.	107	0	0.0
Kahn presumptive	107	0	0.0
Kahn standard	217	3	1.3
Kline diagnostic	122	1	0.8
Kolmer	214	0	0.0
Mazzini flocc.	106	0	0.0

* Based on the 1935 serologic survey and the 1941 Washington serology conference

BIOLOGICAL FALSELY DOUBTFUL AND POSITIVE REACTIONS IN DISEASE AND OTHER CONDITIONS

As previously stated, falsely doubtful and positive reactions with the various serum tests for syphilis have been reported from time to time in a very large number of various diseases and conditions. No useful purpose is served by reviewing the very extensive literature on the subject and particularly the early literature. But even in the case of present-day methods it is difficult to draw hard and fast deductions, largely because of the practical certainty that many of the nonspecific reactions reported have been due to technical factors. Furthermore, with the possible exception of leprosy, it is more or less characteristic of the diseases yielding biologic nonspecific reactions that the true incidence of falsely doubtful or positive reactions may not be elicited by single examinations, especially since they tend to be evanescent and disappear within relatively short periods of time following recovery.

But that falsely doubtful and positive reactions may be due to the presence in sera of a reagin-like or other substance in some diseases, and particularly those due to infections, is now well proved. In Table 7, I have divided these into three groups including a large number in which the evidence is not by any means conclusive at the present time. In this connection it would appear, however, that yaws and pinta may be excluded from the category of biological nonspecific reactions, as the phrase is ordinarily interpreted, because both diseases are due to treponemas either so closely related to *Trepanema pallidum*

TABLE 7

Diseases and Conditions Known or Suspected of Causing Biological Falsely Doubtful and Doubtful Reactions

Group A Variable Incidence	*Group B Incidence Unknown*	*Group C Evidence Not Conclusive*
Yaws	Febrile diseases	Rheumatic fever
Pinta	Upper respiratory tract infections	Glanders
Leprosy	Active tuberculosis	Chancroid (buboes)
Malaria	Septicemia	Vincent's infections
Vaccinia and vaccinoid	Subacute bacterial endocarditis	Rocky Mt. spotted fever
Infectious mononucleosis	Ac. lupus erythem.	Lymphopath. venereum
Virus pneumonia	Relapsing fever	Leishmaniasis
	Rat bite fever	Leukemia
	Weil's disease	Pellagra
	Typhus fever	Psoriasis
	Trypanosomiasis	Coronary thrombosis
		Diabetes mellitus
		Eclampsia
		Lead poisoning
		Acute alcoholism
		Ether anesthesia
		Sulfonamide therapy
		Serum therapy

or, indeed, the latter itself as so stoutly maintained by some investigators in so far as yaws is concerned. The literature on pinta has been thoroughly reviewed recently by Stokes, Beerman, and Ingraham.[8]

per cent, of a group of 65 cases of vaccinia showed falsely doubtful or positive serological reactions as well as 10, or 30 per cent, of a group of 45 cases of vaccinoid. In the majority of instances these reactions were observed

TABLE 8

Falsely Doubtful and Positive Reactions Occurring in Presumably Non-syphilitic Individuals with Various Diseases and Conditions *

	Leprosy (4), (5)		*Malaria (4), (5)*		*Vaccinia (7), (8)*		*Tuberculosis (4), (5), (6)*		*Febrile Dis. (4), (5)*	
Tests	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Eagle Wass.	59	45.7	12	25.0	...	....	59	0.0	46	0.0
Eagle flocc.	110	68.2	45	22.2	...	....	562	0.7	90	1.1
Hinton	60	28.3	48	16.7	263	11.8	562	2.5	89	4.5
Kahn stand.	107	63.5	47	30.0	202	8.9	561	0.5	92	0.0
Kline (diag.)	110	61.8	47	21.3	263	5.3	110	0.9	90	0.0
Kolmer	109	63.3	47	29.8	465	3.9	560	0.16	91	2.2
Mazzini flocc.	58	38.0	12	50.0	465	12.3	59	0.0	46	0.0

* Single examinations

As shown in Table 8, the incidence of doubtful and positive reactions in leprosy as based upon single examinations by author-serologists in the 1935 and 1941 Washington serology surveys, has varied from 38 to 68.2 per cent while the incidence in malaria has varied from 21.3 to 50 per cent. In malaria, however, Kitchen, Webb, and Kupper [19] have reported that serially repeated tests show that 90 to 100 per cent of patients at some time in the course of the disease give biologic falsely positive Wassermann and Kahn reactions.

Since Barnard [20] first reported biologic falsely positive reactions occurring in an individual with vaccinia, the series of cases reported by Lynch and his colleagues [7] and Favorite [8] have together shown an incidence of doubtful and positive reactions varying from 3.9 to 12.3 per cent (Table 8) with the probability that the incidence is higher in flocculation than in complement-fixation tests. In this connection Major Charles R. Rein, Chief of the Division of Serology of the Army Medical School, informs me [21] that 26, or 60

during the second week following vaccination and, with one exception, disappeared within 3 months. Of 28 additional individuals developing immediate or immunity reactions, only 2 showed doubtful or positive reactions. Incidentally, Major Rein also states that very few, if any, soldiers, showed doubtful or positive reactions ascribable to vaccination against tetanus or typhoid fever.

Curiously enough, however, while it is definitely proved that vaccinia and vaccinoid may produce temporary falsely doubtful or positive reactions in human beings, this may not occur in experimental vaccinia of rabbits. At least Miss Rule and I have observed negative Kahn, Kline, and Kolmer reactions in all of a small group comprising 12 normal rabbits with vaccinia in tests conducted 4 days, 1, 2, 3, and 4 weeks after the development of vaccinal lesions of the abdominal skin. All of the animals were selected on the basis of two preliminary negative Kahn, Kline, and Kolmer reactions before inoculation with highly virulent virus, all of the Kolmer tests being conducted

by the modification of my test [22] for avoiding the nonspecific reactions shown by a large percentage of normal rabbits.

It would also appear that pulmonary tuberculosis, especially active febrile cases (Table 8), may show a very low incidence of falsely doubtful or positive reactions [4, 5, 6] as may likewise be true of other febrile diseases [4, 5] and especially the acute exanthemata. Here again, however, Miss Rule and I have observed no confirmatory evidence in our experiments with a small number of rabbits. For example, 12 normal animals were selected on the basis of two preliminary negative Kahn, Kline, and Kolmer reactions. Four were inoculated intradermally (abdominal) with a virulent broth culture of Type I pneumococcus, 4 with a virulent culture of group A hemolytic streptococcus, and 4 with a virulent culture of *Staphylococcus aureus*. Within 48 hours all developed large lesions and fever, 4 succumbing with septicemia (positive heart blood cultures) in 3 to 7 days after inoculation. Kahn, Kline, and Kolmer tests conducted with the sera of surviving animals 4 days, 1, 2, 3, and 4 weeks after inoculation gave consistently negative reactions in all.

However, special mention should be made of falsely doubtful and positive reactions occurring in atypical pneumonias, and especially those due to viruses. Major Rein states [21] that 10 cases in 50 have shown them after the 12th day of the disease, the majority disappearing or reacting negatively in 2 to 6 months.

In afebrile diseases, obstructive jaundice, malignant tumors, menstruation and pregnancy, however, the incidence of falsely doubtful and positive reactions (Table 9) is apparently so nearly the same as may be expected from technical factors that I believe it may be properly stated that these diseases and conditions do not yield biologic nonspecific reactions.

Unfortunately infectious mononucleosis has not been subjected to study in the serologic surveys. Furthermore, the literature is quite contradictory and confusing and fails to indicate the true incidence of falsely doubtful and positive reactions in this disease because it is based so largely upon single examinations. Thus, as shown in Table 10, the incidence of reactions reported by various investigators has varied all the way from none to 100 per cent, with an average of 7.3 per cent doubtful or positive complement-fixation reactions in 202 cases and 9.6 per cent doubtful or positive flocculation reactions in 157 cases.

TABLE 9

Falsely Doubtful and Positive Reactions Occurring in Presumably Non-syphilitic Individuals with Various Diseases and Conditions *

	Afebrile Dis.† (5)		*Jaundice* (4)		*Tumors* ‡ (4), (5)		*Menstruation* (4)		*Pregnancy* (4)	
Tests	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*	*No.*	*Per cent*
Eagle Wass.	131	0.0	..	...	48	0.0	..	...	..	...
Eagle flocc.	131	1.5	49	0	109	0.9	25	0.0	54	0.0
Hinton	131	0.0	51	5.9	110	4.5	24	4.2	53	5.7
Kahn (stand.)	131	0.0	51	1.9	111	0.0	25	0.0	54	0.0
Kline (diag.)	131	0.0	51	0.0	111	0.0	25	0.0	54	0.0
Kolmer	129	0.8	50	2.0	111	0.9	25	0.0	54	0.0
Mazzini flocc.	131	1.5	..	...	49	0.0	..	...	..	...

* Single examinations

† Convalescent pneumonia, diseases of gastrointestinal tract, diabetes, cardiovascular disease, postoperative convalescence and fractures

‡ Malignant neoplastic disease

TABLE 10

Doubtful and Positive Reactions Reported in Infectious Mononucleosis *

Authors	Complement-fixation		Flocculation	
	No. Cases	*No. Doubtful or Positive*	*No. Cases*	*No. Doubtful or Positive*
Parkes-Weber [9]	..	..	3	1
Parkes-Weber and Bode [10]	3	3	3	2
Gooding [11]	27	16	..	..
Butt and Foord [12]	18	0	..	..
Bernstein [13]	37	6	37	4
Fowler and Tidrick [14]	3	2	3	1
Kauffman [15]	82	3	82	1
Mills and Jahn [16]	9	0	9	0
Werlin, *et al.*[17]	4	4	4	4
Kolmer, *et al.*[18]	19	1	16	2
Totals	202	35 (7.3%)	157	15 (9.6%)

* Mostly single examinations

SEROLOGIC INDICES

While it is not my purpose to discuss at length the present-day serologic tests from the standpoint of sensitivity in the serum diagnosis of syphilis, the subject may be referred to briefly. In Table 11 has been summarized the doubtful and positive reactions reported by author-serologists in the nine serologic surveys, including the Washington serology conference, as indicative of what may be expected under optimum conditions with the tests listed. It will be observed that the incidence for all cases of syphilis, treated and untreated, has varied from 77.6 to 90.9 per cent. But in evaluating the various tests due consideration must be given the incidence of falsely doubtful and positive reactions. I have sought to do this in each test by subtracting from the percentage of reactions observed in syphilis (Table 11) the percentage observed in presumably normal non-syphilitic individuals (Table 2) multiplied by 10, as suggested by Herman Brown. The results are what I have termed the "serologic indices" shown in Table 11. On this basis the present-day serologic tests listed in the table show indices varying from 66.5 to 84.5 per cent. Needless to state, it is not reasonable to expect that any serologic test can be made sufficiently sensitive to yield positive reactions in all cases of syphilis under conditions whereby the incidence of falsely doubtful and positive reactions are kept at their present very low

TABLE 11

Doubtful and Positive Reactions Reported by Author-Serologists with the Sera of Syphilitic Individuals in the Serologic Surveys *

Tests	*Total Sera Tested*	*Doubtful and Positive*		*Serologic Indices*
		No. Sera	*Per cent*	
Eagle Wassermann	1,678	1,302	77.6	77.6 — (0.6 × 10) = 71.6
Eagle macroflocc.	1,880	1,544	82.1	82.1 — (1.4 × 10) = 68.1
Eagle microflocc.	1,104	944	85.5	85.5 — (1.9 × 10) = 66.5
Hinton regular	2,281	1,941	85.1	85.1 — (0.5 × 10) = 80.1
Kahn presumpt.	1,855	1,559	84.0	84.0 — (0.3 × 10) = 81.0
Kahn stand.	2,083	1,647	79.1	79.1 — (0.2 × 10) = 77.1
Kline diag.	2,295	1,860	81.0	81.0 — (0.4 × 10) = 77.0
Kline exclusive	1,489	1,353	90.9	90.9 — (1.0 × 10) = 80.9
Kolmer	2,276	1,790	78.6	78.6 — (0.0 × 10) = 78.6
Mazzini flocc.	835	722	86.5	86.5 — (0.2 × 10) = 84.5

* 1935–1943 including the Washington serology conference

and gratifying levels. For example, time is required for the production of reagin in primary syphilis, and its production in chronic latent syphilis may be so slight as to escape serological detection. Undoubtedly, however, further serologic research will improve sensitivity, especially in the way of producing superior antigens, but this is only to be welcomed in case the incidence of nonspecific reactions due to *technic* is kept at or very near zero. It may be, however, that any material increase in sensitivity for syphilis will also increase the incidence of biologic nonspecific reactions in normal individuals and those with non-syphilitic diseases.

SPIROCHETAL COMPLEMENT-FIXATION TEST

While the pioneer investigations on complement-fixation in syphilis employing antigens prepared of cultures of alleged *Treponema pallidum* were conducted in this country about thirty years ago by Noguchi,[23] Craig and Nichols [24] and Kolmer, Williams, and Laubaugh,[25] the subject never attracted much attention until 1929 when Gaehtens and Otto [26] greatly renewed interest in it by reporting that a phenolized saline suspension of cultures of the Reiter strain of alleged *T. pallidum* as antigen, commercially available in Germany under the name of "Palligen" yielded specific complement-fixation reactions in syphilis which were apparently separate and independent of the Wassermann and flocculation reactions as well as more sensitive than the latter, especially in treated syphilis. I have elsewhere summarized the literature on the subject,[27] but here it may be stated that of a total of 13,636 tests conducted with "palligen" and the sera of syphilitic individuals, the incidence of positive reactions has varied from 44.5 to 100 per cent, with an incidence of positive Wassermann reactions varying from 30.4 to 100 per cent. In a total of 36,255 tests conducted with "palligen" and the sera of presumably non-syphilitic individuals the incidence of falsely positive reactions has varied from 0 to 3.4 per cent, with an incidence of 0 to 2.7 per cent in the case of the Wassermann reaction.

In the Washington serology conference [5] complement-fixation tests were conducted by Dr. Eagle with a spirochetal antigen prepared of cultures of the Reiter strain. Carola E. Richter of the Pennsylvania State Department of Health Laboratories, who kindly substituted for me at the conference, also conducted the Kolmer complement-fixation test with an antigen prepared of cultures of the same strain by Clara Kast. The results observed with both tests, as well as in duplicate tests employing the Eagle and Kolmer tissue or lipoidal antigens, are summarized in Table 12.

It will be observed that the two spirochetal complement-fixation tests yielded from 0.6 to 2.9 per cent falsely doubtful and positive reactions with the sera of normal individuals. In the Kolmer tests this percentage was not as high as previously reported [28] because the antigen was used in a smaller amount, but Kolmer and his colleagues [27] [28] have ascribed them to the presence of natural spirochetal antibody in human sera which is apparently of a group character, as similar reactions have been observed with antigens prepared of cultures of *T. microdentium* and *T. macrodentium*.[28] As expected, no falsely doubtful or positive reactions were observed with either test in the examination of cerebrospinal fluids from 107 presumably normal individuals and non-syphilitic individuals afflicted with neurologic or mental diseases, because natural antibodies do not ordinarily occur in normal spinal fluids. But it is evident, as shown in Table 12, that this spirochetal antibody is increased not only in syphilitic but in non-syphilitic

TABLE 12

Percentages of Doubtful and Positive Reactions in Complement-Fixation Tests Employing Tissue and Spirochetal Antigens *

Clinical Status	Total Sera Tested	Eagle Tests		Kolmer Tests	
		Tissue Antigen	Spiro. Antigen	Tissue Antigen	Spiro. Antigen
Normal individuals	169	0.0	0.6	0.0	2.9
Afebrile diseases or conditions	130	0.0	2.3	0.8	6.2
Intercurrent febrile diseases	46	0.0	4.4	2.2	10.9
Tuberculosis (any type)	59	0.0	3.4	0.0	6.6
Malignant disease	49	0.0	8.1	0.0	20.4
Leprosy in any stage	60	45.7	24.1	61.0	28.8
Malaria (febrile and afebrile)	12	25.0	25.0	54.5	27.3
Early syphilis (prim. and sec.)	45	84.1	88.8	91.1	84.4
Syphilis, less than 4 yrs.	119	49.5	61.3	60.5	59.6
Syphilis, over 4 yrs.	212	68.3	90.0	80.0	80.5

* Based upon the results reported at the Washington serology conference

individuals as well with febrile and afebrile intercurrent diseases, tuberculosis, malignant disease, leprosy, and malaria. Under the circumstances it is also evident that the spirochetal complement-fixation test is of no value in differentiating between true and falsely doubtful and positive reactions in so far as serum tests are concerned. It may be otherwise, however, in the case of tests employing cerebrospinal fluid, not only because falsely doubtful and positive reactions apparently do not occur in normal individuals in tests employing spirochetal antigens, but because both Eagle and Kolmer reported about 85 per cent doubtful and positive reactions in 127 treated and untreated cases of syphilis of the central nervous system. Under the circumstances a positive spirochetal complement-fixation reaction with spinal fluid in a doubtful case of syphilis may be of value in establishing the presence of the disease, although a negative reaction is of no value in excluding the possibility of its presence. In this connection it is also to be stated that Kolmer, Kast, and Lynch [29] found that antigens prepared of virulent *T. pallidum* (Nichols-Hough strain) obtained from acute testicular sypilomas of rabbits, were more sensitive and specific than antigens prepared of cultures of alleged *T. pallidum,* but owing to technical difficulties in their preparation they probably cannot be employed.

Undoubtedly, therefore, antibody for *T. pallidum* is produced in syphilis and presumably likewise in yaws and pinta. This is based not only upon complement-fixation but upon agglutination tests as well in so far as syphilis is concerned.[29] Why an increase of spirochetal complement-fixing antibody occurs in leprosy, malaria, tuberculosis, and other diseases listed in Table 11 is difficult to state. It may be assumed that the plasmodia of malaria and the bacillus of leprosy share with *T. pallidum* a common antigenic substance responsible for its production. But unless or until proved otherwise, I prefer to assume that in these non-syphilitic diseases it is due to increase of the natural group spirochetal antibody in the nature of an anamnestic reaction.

MECHANISM OF FALSELY DOUBTFUL AND POSITIVE REACTIONS

Little of a definite nature is known of the mechanism of complement-fixation and flocculation reactions in syphilis employing alcoholic extracts of beef heart or other mammalian tissues as antigens. This is very unfortunate because precise knowledge is so important in relation to the solution of the problem of falsely doubtful and positive

reactions. I believe it may be stated, however, that the mechanism is fundamentally the same in each procedure. At least it appears that both are due to the presence in serum and spinal fluid of a substance capable of flocculating the alcohol-soluble tissue lipoids in colloidal suspension. In complement-fixation tests these flocculi cannot be seen with the naked eye, but are capable of absorbing or fixing complement. But under proper technical conditions the flocculi can be rendered grossly visible, as in the macroscopic flocculation tests, or, microscopically visible by ordinary examinations, as in the microscopic flocculation tests.

The important question concerns the nature of this flocculating substance. This applies not only to syphilitic human beings but also to normal persons and non-syphilitic individuals with other diseases; also to normal rabbits, cattle, horses, mules, chickens, and other lower animals which are well known to yield biological falsely doubtful and positive complement-fixation and flocculation reactions. It is commonly regarded as an antibody or antibody-like substance known as reagin.

In the light of our present knowledge, however. it seems to me that this substance is not an antibody and quite separate and apart from the spirochetal antibody previously discussed. Rather it appears that it may be a modified serum globulin which may not be detected by ordinary chemical methods, although Cardon and his colleagues [34] have suggested recently that hyperproteinemia, and especially hyperglobulinemia, may be in relation to the mechanism of biologic nonspecific reactions. At least various investigators [31–34] have shown that practically all sera of normal persons contain a substance capable of flocculating tissue lipoids under suitable technical conditions. Under the circumstances it appears that the difference between non-syphilitic and syphilitic sera is one of degree rather than of kind—quantitative rather than qualitative.[31] In syphilis, yaws, and pinta it may be that the reacting substance is only an increase of this factor, although I believe that it may be a globulin or a modified globulin which, as previously stated, may not always be detected by the usual chemical methods now employed. If this is true, one may readily understand that an increase of a normal globulin or its modification may account for positive complement-fixation and flocculation reactions not only in syphilis, yaws, and pinta, but in an occasional normal person as well as in non-syphilitic individuals with leprosy, malaria, vaccinia, infectious mononucleosis, virus pneumonia, etc.

I have elsewhere discussed this subject with more detail [27] but here it may be stated that the preponderance of evidence at the present time is in favor of regarding the reagin as separate and distinct from the spirochetal antibody. Gaehtgens [35] as well as Kroó, Schultze, and Zander [36] have expressed this opinion while Beck,[37] Kolmer, Kast, and Lynch [38] have come to this conclusion largely on the basis of absorption tests with syphilitic sera with tissue lipoids for the removal of the reagin and with spirochetes for the removal of the spirochetal antibody. Eagle and Hogan,[39] however, have stated on the basis of their absorption experiments, that the lipodotropic reagin and spirochetal antibody are identical, or, in other words, that positive complement-fixation and flocculation reactions with tissue antigens as well as positive spirochetal complement-fixation reactions are due to spirochetal antibodies.

Be this as it may, the important practical question is whether or not the reagin responsible for biological nonspecific reactions with the sera of human beings and the lower animals is different in its properties from the reagin

responsible for complement-fixation and flocculation reactions in syphilis, yaws, and pinta. Kahn [40] believes that the former is most active in flocculation tests conducted at a cold temperature (1° C.) and when the salt concentration of the reacting system is reduced to a minimum, while the latter is most active at body temperature. On this basis he has devised various " verification tests " for differentiating between biological nonspecific and specific reactions to which further reference will be made. Here it may be stated, however, that in the Kolmer complement-fixation test, at least, there can be no doubt of the fact that the reagin produces much more complement-fixation at a cold temperature of 4 to 8° C. over a period of 15 to 18 hours than in a water bath at 38° C. for 1 hour, regardless of whether it is occurring in the normal sera of human beings or the lower animals, in the sera of non-syphilitic human beings with various diseases and conditions, or in the sera of syphilitic human beings. In other words, differentiation between biological nonspecific and specific reactions has not been found possible on the basis of the temperature employed in conducting the tests in so far as complement-fixation is concerned.

Personally, however, I am not without hope that a solution of the problem will be found by changing the methods employed in the preparation of tissue antigens. Investigations now being conducted by Herman Brown and the writer indicate that it may be possible to remove certain lipoids with which the nonspecific reagin reacts without reducing the sensitivity of the antigen in relation to the syphilis reagin. Furthermore, if it is found possible to prepare antigens which are satisfactory for the serodiagnosis of syphilis without the addition of a sensitizing sterol, like cholesterol, an additional step toward a solution of the problem will have been accomplished, and I am not without hope that this is possible.

MANAGEMENT OF FALSELY DOUBTFUL AND POSITIVE REACTIONS

While these matters are being ironed out by present and future serological investigations, however, the management of falsely doubtful and positive reactions occurring under present conditions constitutes a practical problem of first rate importance in order to reduce to a minimum the regrettable error of the needless treatment for syphilis. To this end the close coöperation of clinicians and serologists is required. Even though the incidence of nonspecific reactions due to technic is very low, when present-day approved tests are correctly conducted, and even though false reactions with the sera of normal, presumably non-syphilitic, individuals may occur only once in anywhere from 1,000 to 4,000 examinations, yet all figures and statistics are of little avail in so far as the individual involved is concerned.

As summarized in Table 13, certain clinical procedures are indicated. It is a mistake for the physician to place the whole burden on the laboratory. Certainly no director of a laboratory, serologist or technician who is completely ignorant of the clinical status of the individual and the conditions surrounding the collection, labelling, and delivery of the specimen, can make so important a decision as to whether syphilis is or is not present, whether treatment should or should not be given or, in the case of the latter, the kind and duration of treatment to be administered. In other words, the serologic tests alone are insufficient and do not constitute a short-cut or royal road in differentiation between true and false doubtful and positive reactions. As a matter of fact, the difficulty in the interpretation and management of presumably nonspecific reactions is in

TABLE 13

Clinical Procedures in the Interpretation and Management of Presumably Falsely Doubtful and Positive Reactions

1. Collect and deliver specimens with due care against bacterial and chemical contamination to avoid technical nonspecific reactions (see Table 1).
2. A negative history for syphilis is of little or no value in excluding the possible presence of the disease.
3. Make thorough examinations for acquired and congenital syphilis.
4. Take careful history and make necessary examinations if and when necessary for the diseases and conditions capable of yielding biological nonspecific reactions (see Table 7).
5. Examine spinal fluid in selected cases. Positive results are indicative of syphilis. Negative results do not exclude its possible presence.
6. Provocative treatment may be tried in some cases but negative serologic results do not exclude the possibility of syphilis.

relation to the clinical skill and experience of the physician in chronic acquired and especially late congenital syphilis. There is no substitute for thorough clinical examinations so skillfully indicated and outlined by Stokes and Ingraham [41] and by Moore and his colleagues.[42]

On the other hand, however, serologists and technicians cannot escape participation and a heavy responsibility in the elucidation of the problem in individual cases as I have attempted to summarize in Table 14. Certainly there is no one best test for syphilis. Serological diagnosis is always best served by using two or more approved methods routinely of which, in my opinion, one at least should be a complement-fixation procedure. Needless to state, this is not always possible when very large numbers of tests are to be conducted, as in state and other laboratories. Under such conditions a screen test of acceptable sensitivity and specificity is required, of which my preference is for one of the microflocculation procedures. But I believe that all positive reactions should be checked by another method before a report is rendered. Under these conditions it is inevitable that a screen test may occasionally give a falsely negative reaction, but unless conditions permit the routine use of two or more methods it is about the best that a hard worked laboratory can do. In my own laboratory all sera are tested routinely by a complement fixation, a macroflocculation and a microflocculation procedure.

As previously stated, physicians should never jump to the conclusion that syphilis is present on the basis of a single doubtful or positive reaction unless there is supporting historical or clinical evidence. But, on the other hand, they should not be ignored simply because clinically unsuspected. When such reactions are suspected of

TABLE 14

Serological Procedures in the Interpretation and Management of Presumably Falsely Doubtful and Positive Reactions

1. Conduct the test or tests with care and skill exactly as described by author-serologists to avoid technical nonspecific reactions as much as possible (see Table 1).
2. Conduct the tests by several methods in the same or different laboratories.
3. Spirochetal complement-fixation reactions with *sera* are of no value but positive reactions with *spinal fluids* are indicative of syphilis; negative reactions do not exclude its possible presence.
4. The value of verification tests is uncertain but they are worthy of trial.
5. Withhold judgment and treatment but repeat the tests at intervals over a period of at least 3 to 6 months. If negative reactions occur syphilis is usually to be excluded; if positive reactions persist syphilis is probably present and treatment is advisable.

being nonspecific, judgment and treatment should be withheld for at least 3 to 6 months, with a repetition of the tests every 2 to 4 weeks. If, during that time, the reactions have become repeatedly negative and there are no historical or clinical evidences of syphilis, it would appear that the disease may be excluded. However, if persistently positive reactions are observed with approved methods properly conducted, even though but weakly positive, I see no escape from the advisability of making a tentative diagnosis of syphilis and instituting treatment in the best interests of the individual involved.

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Discussion

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THE concluding paragraph of Dr. Kolmer's paper conveys a thought which may well deserve further emphasis, especially in regard to the transposition of test findings into terms of clinical syphilis. Almost from the time of the development of the Wassermann test a positive finding has been accorded a diagnostic weight amounting almost to infallibility. As the newer tests were developed and came into use they were permitted to share in this position of reliability. As the use of the tests became more general and as the multiple testing of individual sera was practised, the vagaries and discrepancies of test findings became apparent in treated syphilis as well as in conditions without syphilis as a causative factor.

At the present time, and assuming that the methods which are available are carried out in an acceptable manner, the advisability of exercising a careful scrutiny of all factors involved, before permitting the diagnosis of syphilis to be based entirely upon serologic findings, is accepted. This is important especially when clinical and historical confirmations are lacking. The waiting period, referred to by Dr. Kolmer, during which the diagnosis is withheld although positive test findings may have been recorded, serves as a safeguard in instances in which such conditions as infectious mononucleosis, virus pneumonia, vaccinia, or any of a host of other intercurrent factors may be responsible for the positivity expressed by one or a group of well conducted tests. A time interval of several months before making an arbitrary diagnosis is sometimes sufficient for the reacting substance to disappear entirely from the blood serum or to have been reduced in concentration to a point that the decline in strength of the reaction is obvious.

The use of the waiting period is of limited helpfulness in instances of false positive or discrepant findings occurring in pregnancy. Here it becomes necessary to take into account the risk of an unfavorable outcome as regards the child. This consideration sometimes dictates that the patient be placed under treatment without appreciable delay and without too much regard for the refinements of diagnosis. An attempt to establish the true syphilis status of the mother should be made after delivery although the interposition of treatment always complicates the diagnostic problem.